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VALIDATION OF HUMAN EYE IRRITATION BY TEAR LYSOZYME LEVELS

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Tear samples were collected from 358 volunteers in Southern California in smog polluted areas. No consistent variation of lysozyme concentration with oxidant concentration was demonstrated. Subjects exposed to .19 ppm of oxidant in the open air did show decreased lysis. A threshold effect is possible. No correlation was established between lysis and mild subjective complaints of irritation. Objective indications of eye irritation show a mild decrease in lysis levels.

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Purpose of Contract

Eye irritation is a common non-specific complaint of our country's urban population. Previous subjective studies suggested that acrolein, formaldehyde, peroxyacetyl nitrate (PAN), and perhaps the oxides of nitrogen are the components of photochemical smog responsible for eye irritation.[1,2,3,4,5,6,7] Although good correlations between repeated subjective observer responses and oxidant concentrations have been found, these studies are open to criticism as the subjects were well aware that their eye irritation was being assessed by those desiring to control air pollution.[8] Human tear lysozyme has been said to decrease in subjects having acute eye irritation and in various ocular disorders.[11] During the preceding year, we set up a method of measuring human tear lysozyme concentration and analyzed variables such as age, sex, race, temporal factors and the presence or absence of eye irritation. Only age was found to have a significant effect on normal persons. Eye irritation such as from corneal ulcers has been known for many years to cause a decrease in lysozyme concentration.[9]

During the current year we set up a field study in Los Angeles and sampled volunteers in low, medium, and high air pollutant locations to determine the feasibility of using tear lysozyme levels as an objective indicator of eye irritation.

Methods

The field study was conducted in Los Angeles and Riverside Counties during the Week August 31, 1971 through September 3, 1971. Through the

assistance of Dr. Louis Mahoney of the Los Angeles Health Department, tear sampling was done at specific health department clinics which were near air pollution monitoring stations where ambient pollutant samplings have revealed consistent levels. Dr. Pietsch, Dr. Pearlman, and Mrs. Reed arrived in Los Angeles the day prior to sampling with all necessary equipment including 1500 plates for lysozyme analyses.

A total of 358 volunteers were easily found at the health clinics, local police and fire stations and at the County Health Department in Riverside (with Dr. Erickson's assistance). Permission slips were signed prior to sampling by each volunteer and questionnaires were completed (see attachment #1). Volunteers were specifically asked their age, length and place of residence, number of hours out of doors, use of air conditioning, presence of subjective eye irritation and use of eye medications. All eyes were examined for evidence of objective irritation prior to sampling.

The method of Bonavida and Sapse was used for all lysozyme determinations.[10] Topical anesthesia was not used. Tear strips were placed beneath the medial 1/3 of the lower lid of each eye and allowed to remain for five minutes. The moistened 5 mm strips were immediately incubated at 37°C for 24 hours, following which the zone of lysis was measured. Ambient air pollutant levels were monitored in each area during the time of our sampling. Statistical analyses were completed on the data obtained during the remainder of the year.

Results

Overall Effects

The overall average lysis in each area was taken as an index of the chronic effect of oxidants on lysozyme concentration (Table 1). In the areas of high pollutant exposure, Azusa and Riverside, mean oxidant levels during an eleven hour period during the day were only .13 ppm while hourly maxima reached only 0.24 ppm. Although there was a tendency for lower mean lysis in the high oxidant exposed groups, this was not statistically significant. Mean age was similar in all populations sampled ranging from 27.2 years in the control to 29.2 years in Azusa to 36.2 years in Riverside. Mean length of residence was also comparable ranging from 10.8 years in the control to 15.6 years in Azusa to 12.9 years in Riverside. A correlation of average lysis with age or with hourly oxidants, carbon monoxide, and oxides of nitrogen and sulfur revealed no relationship.

Overall mean lysis was 29.0 mm for those with residence less than two years and 29.1 mm for those with residence greater than two years. This difference was not statistically significant. When individual groups were compared, no significant difference existed between them. In a correlation matrix (Table II), no relationships appeared between age and lysis and pollutant concentrations for those with residence of two or more years.

Acute Effects

A regression analysis was performed on all groups using mean lysis as the dependent variable. Time of measurement, age, length of residence,

length of time spent outdoors, and hourly oxidant were the independent variables. Time of measurement was significant for the high exposure group in Riverside and the low exposure group in Los Angeles. In a correlation matrix, mean lysis could not be correlated with oxidant concentration one or two hours previous to sample collection. In addition, mean lysis did not correlate with carbon monoxide, oxides of nitrogen, or sulfur oxide measurements for the day of collection.

Because of the correlation with time, a comparison of subjects measured in the morning with subjects measured in the afternoon was made. Average mean lysis was 28.8 mm in the morning versus 29.2 mm in the afternoon. In the morning group any oxidant reading--maximum oxidant, oxidant two hours prior, oxidant one hour prior, or oxidant at the time of the test--were correlated with lysis. The positive correlation was significant at the 0.05 level. Overall mean lysis and oxidant concentrations at the time of one or two hours previous to the sample collection do not correlate in the correlation matrix. Average age was 33.3 years for the morning subjects and 27.5 for the afternoon subjects. Average residence was 14.5 years for the morning subjects and 11.8 years for the afternoon subjects.

The effects of being indoors and having air conditioning were evaluated. People working in non airconditioned buildings were considered to be "outside" for the purposes of this study. However, since an enclosed area might provide some protection from pollutants, several groups working outside were analyzed separately (Table III). One group of firemen in the high exposure area with the highest oxidant concentration exposure of .19ppm had a mean lysis of 27 mm. This group was also subject to high oxidant exposure for the two hours preceeding testing: .16 and .12 ppm. Another

outside group measured the preceeding hour exposed to .16 ppm at the time of testing and .12 and .08 ppm in the two preceeding hours demonstrated 29 mm of lysis.

Two groups who had been mostly in buildings rather than workir outside, exhibited 29.4 and 29.8 mm of lysis despite exposure to .21 and .20 ppm at the time of testing and the aforementioned high levels in the preceeding hours.

Effects of Air Conditioning

The chronic effect of air conditioning on mean lysis was investigated by selecting all volunteers sampled with air conditioning in both home and office. Forty-six eyes with a mean lysis of 29.0 mm were found in Azusa (high exposure) while 103 eyes with a mean lysis of 28.9 mm were found in Riverside (high exposure). Four eyes in the control group had a mean lysis of 29.1 mm. Conversely, those with no air conditioning in their home or office had 28.8 mm of lysis in Azusa, 29.0 mm in Riverside, and 29.2 mm in the control area. No effect of air conditioning in lysis could be demonstrated.

The effect of subjective and objective evidence of eye irritation on lysis levels was evaluated (Table IV). The subjective presence of eye irritation had no effect on lysis levels. In fact, mean lysis was slightly higher in this group (29.1 mm). No subjects complained of severe eye irritation. Those with objective evidence of eye irritation (infection or mild conjunctivitis) had only slightly lower lysis as one would anticipate (28.69 mm). Mean lysis was higher (28.89 mm) among those with neither subjective nor objective irritation. Table V reveals that the prevalence of volunteers who appeared irritated or appeared and felt irritated was lower in the control area.

Mean lysis was not lower among those working outside complaining of subjective and objective eye irritation (Table III). Only in the previously mentioned group of 18 firemen in Azusa was lower mean lysis found among the four subjects complaining of irritation, 26.3 mm, and the two subjects with subjective eye irritation, 26.5 mm.

Discussion

Bonavida and Sapse reported lower tear lysozyme levels in patients complaining of eye irritation at oxidant concentrations of .15 to .25ppm[11]. In attempting to confirm such findings, our results are disappointing. Overall lysis was not significantly lower in our high exposed group. However, mean daily pollutant concentrations in Azusa and Riverside did not reach the levels Sapse and Bonavida encountered in their report of susceptible persons. A further study done during a smog alert might be more helpful in elucidating the value of the lysozyme technique.

No chronic differences in lysis could be demonstrated between those living in air conditioned areas most of the time and those without this convenience. In addition, subjects measured in air conditioned buildings in Riverside did not show different lysozyme levels than those outside.

Lysis levels were somewhat lower in subjects having both subjective and objective evidence of eye irritation. Any cause of prolonged eye irritation lowers tear lysozyme levels.[9] Perhaps Bonavida and Sapse selected a population of susceptible individuals with measurable differences not present on a day to day basis in the population at large.

It should be emphasized that there was no correlation between only subjective complaints and decreased lysis.

A threshold effect must be considered. Firemen exposed to .19 ppm of oxidant did show lower mean lysis. However, other firemen exposed to .16 ppm exhibited mean lysis similar to that among controls. This may represent a threshold effect. Either the preceding three hours of high oxidant levels or the hourly oxidant level reached could have triggered lower lysozyme levels. For this relationship to be valid, one must assume that the higher mean lysis among the group exposed to .20 ppm was due to a protective effect from being indoors even without air conditioning. A subjective study by Richardson and Middleton[12] suggested a threshold of oxidant concentration of .10 ppm for "barely noticeable" irritation, .18 ppm for "moderate irritation", and .25 ppm for "severe irritation". However, Hammer et al[13] noted no increase in subjective complaints on a day when an oxidant level of .23 ppm was reached.

Further information might be obtained by training several field technicians in the Los Angeles area to collect tear samples. A short study of subjects out of doors during a smog alert could verify or disprove the value of lysozyme assays as an objective index of eye irritation. Higher levels of oxidants might be necessary to get a lysis effect.

Summary

Tear samples were collected from 358 volunteers in Southern California in smog polluted areas. No consistent variation of lysozyme

concentration with oxidant concentration was demonstrated. Subjects exposed to .19 ppm of oxidant in the open air did show decreased lysis. A threshold effect is possible. No correlation was established between lysis and mild subjective complaints of irritation. Objective indications of eye irritation showed a mild decrease in lysis levels.

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ATTACHMENT I

Name _____

Address _____

_____

Location: Max Med Min

Date: __/__/__

Time: 8 - 9 - 10 - 11 - 12 - 1 - 2 - 3 - 4

Age: ____

How long have you been outdoors or in an open building today? ____ hours

Air conditioned: Home: Yes ___ No ___
Office: Yes ___ No ___

How long have you lived in this area? ____ years ____ months _____

Do you often take vitamins? Yes ___ No ___

Do you use eye drops? Yes ___ No ___

If yes, type and time of last drop: _____

Eyes irritated? Yes ___ No ___

Length of time: ___ Less than 1 hour
___ 1 hour - 1 day
___ 1 - 5 days
___ Chronically

Eyes appear irritated: Yes ___ 1+ red No ___
___ 2+ tear
___ 3+ red and tear

Comments regarding eye disease (other than glasses) _____

_____

LYSIS: O.D. _____ mm.
O.S. _____ mm.

WETTING: O.D. _____ mm.
O.S. _____ mm.

Table I

Mean Lysis vs Maximum Pollutant Levels

Sample Station	Mean Lysis	Daily Maximum Pollutants				Oxidant (11 hrs.)
		Ozone (ppm)	CO	Nitric Oxide	Sulfur Oxides	
Azusa, 8/30	28.71	0.16	6	.22	.04	.08
Riverside, 8/31	28.98	0.24	-	-	-	.13
Azusa, 9/1	28.76	0.23	7	.24	.05	.13
Inglewood Imperial Heights, 9/2	29.17	0.02	9	.30	.04	.01
Inglewood, 9/3	29.23	0.04	15	.47	.06	.01

Table II

Correlation Coefficients of Mean Lysis with Other Variables

(Correlation of .42 is Significant)

Factor	All Participants	Azusa & Riverside (High Exposure)
Time	.10	.23
Age	-.13	-.19
Oxidant 2 hours before	-.01	.24
Oxidant 1 hour before	-.03	.14
Oxidant at time of sample collection	-.03	.16

Table III

Summary of Outdoor Group

Group Sampled		Number Eyes Sampled	Oxidant	Mean Lysis	Number with Subjective Irritation	Mean Lysis	Number with Objective Irritation	Mean Lysis
Azusa	8/30 11 a.m.	22	.10	28.8	1	28.0	1	30.0
Azusa	8/30 3 p.m.	18	.14	29.4	4	29.3	5	29.6
Azusa	8/30 4 p.m.	8	.11	28.5	0	-	0	-
Riverside	8/31 12 p.m.	23	.13	28.96	6	28.8	5	29.2
Azusa	9/1 11 a.m.	20	.16	29.0	1	29.0	1	28.5
Azusa	9/1 12 p.m.	18	.19	27.0	4	26.3	2	26.5

Table IV

Effect of Subjective and Objective Eye Irritation on Lysis

Date	Exposure Level	Lysis Levels				Neither S nor O
		Mean	S	O	S+O	
8/30	High	28.71	28.92	28.71	29.11	28.64
8/31	High	28.98	28.97	28.17	29.17	28.93
9/1	High	28.76	28.25	28.20	26.50	29.11
9/2	Low	29.17	29.22	28.45	--	29.21
9/3	Low	29.23	29.31	29.08	30.0	29.24
Overall	-	28.95	29.13	28.72	28.69	28.89

1 = Subjective eye irritation
2 = Objective eye irritation
3 = Both subjective and objective evidence of eye irritation

Table V

Prevalence of Eye Irritation Among Subjects Sampled (per cent)

Sample Station	Appeared Irritated	Felt Irritated	Looked & felt Irritated
Riverside	16.1	28.4	10.9
Azusa	29.3	21.0	9.1
Imperial Heights (control)	5.9	18.1	0.9
Overall	29.6	45.3	13.7